Production of Cattle Immunotolerant to Bovine Viral Diarrhea Virus

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ABSTRACT

Inoculation of bovine virus diarrhea virus into 58 to 125 day old fetuses of bovine virus diarrhea virus seropositive pregnant cows, or inoculation of bovine virus diarrhea virus into seronegative cows 42 to 114 days pregnant, may produce clinically normal calves which are persistently infected with the specific isolate of bovine virus diarrhea virus yet seronegative to the homologous and heterologous isolates. Reinoculation of these persistently infected cattle with their homologous isolate produced no neutralizing antibody response to bovine virus diarrhea virus. These persistently infected cattle were immunocompetent as they developed neutralizing serotiters to infectious bovine rhinotracheitis, parainfluenza-3 viruses and agglutinating serotiters to *Pasteurella hemolytica*.

Key words: Immunotolerance, bovine viral diarrhea (BVDV), intrauterine infection, BVDV in the fetus.

RÉSUMÉ

L'inoculation du virus de la diarrhée à virus bovine à des foetus âgés de 58 à 125 jours, dont les mères possèdent déjà des anticorps contre ce virus, ou à des vaches dépourvues de tels anticorps et qui comptent de 42 à 114 jours de gestation, peut produire des veaux apparemment normaux qui demeurent constamment infectés par la souche de virus qu'on leur a inoculée, mais séronégatifs à l'endroit de cette souche ou de souches hétérologues. La réinoculation éventuelle avec la souche qu'ils avaient reçue avant leur naissance ne provoqua pas la formation d'anticorps contre le virus de la diarrhée à virus bovine. Ces bovins infectés de façon permanente se révélèrent toutefois immunocompétents, puisqu'ils développèrent des anticorps neutralisants à l'endroit du virus de la rhino-trachéite infectieuse bovine et de celui du parainfluenza #3, ainsi que des anticorps agglutinants à l'endroit de *Pasteurella haemolytica*.

Mots clés: immunotolérance, diarrhée à virus bovine, infection intra-utérine, virus de la diarrhée à virus bovine chez le foetus.

INTRODUCTION

Several apparently healthy Holstein-Friesian cows that carried the bovine viral diarrhea virus (BVDV) were found to be seronegative for the virus (1). Also one bull calf, seronegative for BVDV, carried virus in the precolostral buffy coat cells and the synovial fluid of the hock joints. The animals that carried virus but did not develop neutralizing antibodies were considered to be persistently infected with BVDV. The persistently infected animals were apparently healthy newborn calves which lived to breeding age (1). Steck *et al* have also found cattle that were infected with BVDV but which developed no antibody (2).

This raised questions of when and under what circumstances infection took place, and whether more than one isolate of BVDV could cause this condition. It seemed probable that infection took place at some stage of gestation, perhaps before the fetus became immunocompetent. To study these questions, five experiments were undertaken using five different isolates. Two BVDV isolates were inoculated into 17 fetuses of BVDV seropositive cows at 58 to 125 days of gestation, and three BVDV isolates were inoculated intravenously into 44 BVDV seronegative cows at 42 to 114 days of gestation.

MATERIALS AND METHODS

The BVDV isolates used in these experiments were propagated on bovine turbinate cells (3). Isolate 7443, isolated from a persistently infected calf (1), was used at the fifth cell culture passage level and the titer was $10^{4.1}$ $TCID_{50}$ per 0.1 mL. The VM isolate, isolated from a stunted chronically infected yearling animal, was used at the tenth cell culture passage level and the titer was $10^{4.3}$ $TCID_{50}$ per 0.1 mL. The cell culture passage level of the New York-1 (NY-1) isolate was unknown, but probably at least the 20th passage and the titer was 10^5 $TCID_{50}$ per 0.1 mL. The cell culture passage level of the NADL isolate was unknown, but probably above 30 passages and the titer was 10^6 $TCID_{50}$ per 0.1 mL. The MC isolate, isolated from an unthrifty persistently infected yearling seronegative for BVDV, was used at the ninth cell culture passage level and the titer was $10^{4.0}$ $TCID_{50}$ per 0.1 mL. The NADL isolate was cytopathic, the NY-1 isolate was noncytopathic, and the 7443 and VM isolates were basically noncytopathic, but a few vacuoles would appear in the cell culture. The initial isolation of the MC isolate was noncytopathic; but after the fifth passage, an occasional passage of the virus would develop CPE after seven to ten days incubation.

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Pregnancy of all cows used in these experiments was determined by palpitation of the gravid uterus 40 days or later after breeding. In the first experiment, laparotomy was performed on 12 BVDV seropositive cows at 58 to 125 days of gestation and 5 mL of BVDV isolate number 7443 was injected into the amnionic fluid. In the second experiment, 20 mL of VM isolate was inoculated intravenously into six virus negative and seronegative cows from 49 to 84 days of gestation. In the third experiment 5 mL of (NY-1) BVDV was inoculated into the amnionic fluid, as in the first experiment, of five fetuses of seropositive cows at 87 to 98 days of gestation. In the fourth experiment, 10 mL of NADL BVDV was inoculated intravenously into seven virus negative and seronegative heifers at 75 to 114 days of gestation. In the fifth experiment, 20 mL of MC BVDV was inoculated intravenously into 15 virus negative and seronegative heifers at 42 to 112 days of gestation.

The cows were allowed to calve and precolostral serum and buffy coat samples were taken for determination of the presence of antibody or virus. The calves were allowed to nurse their dams for four months, then weaned. The calves and their dams were held in outside holding pens in groups of four to six animals and subjected to natural climate and cattle-raising conditions.

Selected tissues for immunofluorescent antibody staining (IA) were taken from some of the calves that died and which were exposed to 7443, VM and MC isolates. Not all tissues were examined from each animal. Adrenal gland, aorta, brain, heart, intestine, kidney, liver, lung, mediastinal lymph node, mesenteric lymph node and spleen were imbedded in 2.5% methyl cellulose, frozen, and sectioned at 6 μm; sections were fixed in acetone and stained directly with IA to BVDV (4). Specificity of the reaction was evaluated by blocking positive tissues with homologous unlabeled antiserum and by staining equivalent tissue sections from noninfected control animals.

To evaluate immunocompetency, four 12 month old persistently infected bulls (8798, 8914, 8915, 8916) from experiments 1 and 2 were exposed by intravenous injection of 10 mL bovine rhinotracheitis virus (IBR) ($TCID_{50}$ $10^{6.8}$ per 0.1 mL), then 30 days later 10 mL parainfluenza-3 virus (PI-3) ($TCID_{50}$ $10^{5.8}$ per 0.1 mL). Thirty days later these four bulls and bulls 9132 of Table III and 9133 of Table I were then given 10 mL of their homologous BVDV intravenously. Thirty days later bulls 8798, 8914, 8915 and 8916 were forced to breathe an aerosol suspension of an 18 hour broth culture (2 x 10^9 colony forming units) of *Pasteurella hemolytica* for 20 minutes.

RESULTS

The results of the first experiment, Table I, indicated persistently infected calves were produced by infecting fetuses from 58 to 125 days of gestation. However, clinical response to fetal inoculation varied, resulting in: abortion of four fetuses; stillbirth of a full term calf; birth of a weak calf that had to be hand fed for two days; birth of five apparently normal calves which were persistently infected with BVDV, but were seronegative to the virus; and the birth of one normal calf (inoculated at 125 days of gestation) which was seropositive for BVDV.

The weak calf (8870, Table I) grew well and looked thrifty until a month after weaning when she developed a pyrexia and acute diarrhea and died. Necropsy revealed a marked hemorrhagic ileitis approximately 40 cm in length, but no other gross lesions. Another normal appearing heifer (8917) developed chronic pneumonia after weaning and died a month later.

The results of the second experiment, Table II, indicated persistently infected calves were also produced by inoculating seronegative cows from 49 to 84 days of gestation with the VM isolate. The six cows gave birth to persistently infected calves which appeared to be in good health. All calves grew normally until near weaning, then two calves (8909 and 8912)

TABLE I. Calves Persistently Infected With 7443 Isolate of Bovine Viral Diarrhea Virus (BVDV) by the Injection of the Fetuses of Cows Seropositive for BDV

Cow Number	Calf Number	Day of Gestation Inoculated	Appearance of Calf at Birth	Virus Isolated	Sero Status for BVDV at Birth	Disposition of Calf
1	—	81	No calf	—		
2	—	105	No calf	—		
3	8798	58	Normal-vigorous	Yes	Neg	Normal yearling — used on another experiment
4	8799	125	Normal-vigorous	Yes	Neg	Normal two year old — bred and had a normal appearing persistently infected calf, 9133, and a second normal appearing infected calf, 9310
5	8870	90	Weak	Yes	Neg	Normal and grew well for four months then developed acute diarrhea and died
6	8875	105	Normal-vigorous	Yes	Neg	Normal two year old — bred. Had a clinically normal persistently infected calf
7	—	99	No calf	—		
8	—	100	No calf	—		
9	8910	125	Normal-vigorous	No	Positive	Normal yearling — used on another experiment
10	8913	103	Stillborn	Yes	Neg	
11	8914	103	Normal-vigorous	Yes	Neg	Normal yearling — used on another experiment
12	8917	90	Normal-vigorous	Yes	Neg	Developed chronic pneumonia after weaning and died in a month

TABLE II. Calves Persistently Infected With VM Isolate of Bovine Viral Diarrhea Virus (BVDV) by Intravenous Inoculation of Pregnant Cows Seronegative for BVDV

Cow Number	Calf Number	Day of Gestation Inoculated	Appearance of Calf at Birth	Virus Isolated	Sero Status for BVDV at Birth	Disposition of Calf
13	8908	79	Normal-vigorous	Yes	Neg	Normal seven month old calf killed in an accident
14	8909	84	Normal-vigorous	Yes	Neg	Calf developed chronic diarrhea and was in poor condition with pot belly and swollen submaxillary lymph glands. Died at four months of age
15	8911	79	Normal-vigorous	Yes	Neg	Normal two year old — bred, but lost the calf and developed acute mucosal disease at two years and four months of age
16	8912	72	Normal-vigorous	Yes	Neg	At three months of age calf developed chronic diarrhea and chronic pneumonia and died
17	8915	49	Normal-vigorous	Yes	Neg	Normal yearling — used on an experiment
18	8916	49	Normal-vigorous	Yes	Neg	Normal yearling — used on an experiment

developed chronic diarrhea and died.

The results of the third experiment, Table III, indicated NY-1 BVDV could also produce persistently infected calves when fetuses were injected at 87 to 98 days of gestation. There was one abortion six weeks after fetal infection, two other abortions at sometime during gestation and two apparently normal persistently infected seronegative calves born. The bull calf grew well and appeared normal at approximately 12 months of age, but the heifer has remained rather small and unthrifty.

The results of the fourth experiment, Table IV, indicated NADL BVDV was different in that it crossed the placenta and infected the fetuses, but did not produce any persistently infected calves. There was one abortion, but six apparently normal calves were born, all with antibody to BVDV, but virus was not isolated from the buffy coats.

The results of the fifth experiment, Table V, indicated the MC isolate could also cross the placenta and produce persistently infected calves when pregnant cows were inoculated at 43 to 112 days of gestation. However, from this group of cattle, there were only six apparently healthy persistently infected calves growing at eight months of age.

DETERMINATION OF IMMUNOCOMPETENCE

The four persistently infected yearling bulls exposed to IBR and PI-3 developed neutralizing antibodies that rose from a titer $< 1{:}4$ to $> 1{:}128$ against each virus. None of the six bulls injected with their homologous BVDV isolate developed a serotiter to BVDV.

One bull (8916) was very wild and underwent considerable stress during

TABLE III. Calves Persistently Infected With New York-1 Isolate of Bovine Viral Diarrhea Virus (BVDV) by the Injection of the Fetuses of Cows Seropositive for BVDV

Cow Number	Calf Number	Day of Gestation Inoculated	Appearance of Calf at Birth	Virus Isolated	Sero Status for BVDV at Birth	Disposition of Calf
19	—	90	Aborted	No		
20	—	90	Aborted	ND		
21	—	87	Aborted	ND		
22	9131	98	Normal-vigorous	Yes	Neg	Not thrifty and small, used on another experiment
23	9132	87	Normal-vigorous	Yes	Neg	Appears normal yearling, used on another experiment

ND = not done

TABLE IV. Calves Infected With NADL Isolate of Bovine Viral Diarrhea Virus (BVDV) by Intravenous Inoculation of the Pregnant Cows Seronegative for BVDV

Cow Number	Calf Number	Day of Gestation Inoculated	Appearance of Calf at Birth	Virus Isolated	Sero Status for BVDV at Birth	Disposition of Calf
24	9134	114	Normal-vigorous	No	1:64	Apparently normal yearling
25	9135	108	Normal-vigorous	No	1:128	Apparently normal yearling
26	9136	88	Normal-vigorous	No	1:16	Apparently normal yearling
27	9137	87	Normal-vigorous	No	1:256	Apparently normal yearling
28	9139	86	Normal-vigorous	No	1:2	Apparently normal yearling
29	—	98	Returned in heat in 60 days			

exposure to the aerosol of *P. hemolytica*. It developed pyrexia of 40°C within 24 hours of exposure and died of acute pasteurellosis in three days. The three other bulls exposed to *P. hemolytica* developed pyrexia of 40°C within 48 hours, then made an uneventful recovery. Following exposure to *P. hemolytica*, the indirect hemagglutinating titer of 8798 rose from 1:4 to 1:64, 8914 from 1:16 to 1:128, and 8915 from 1:32 to 1:512.

IMMUNOFLUORESCENCE

The BVDV antigen was most consistently seen in the glomeruli (Fig. 1) and collecting tubules (Fig. 2), and in some parts of the intestine (Fig. 3) by IA. However, the antigen was widespread in many tissues of some infected animals, Table VI.

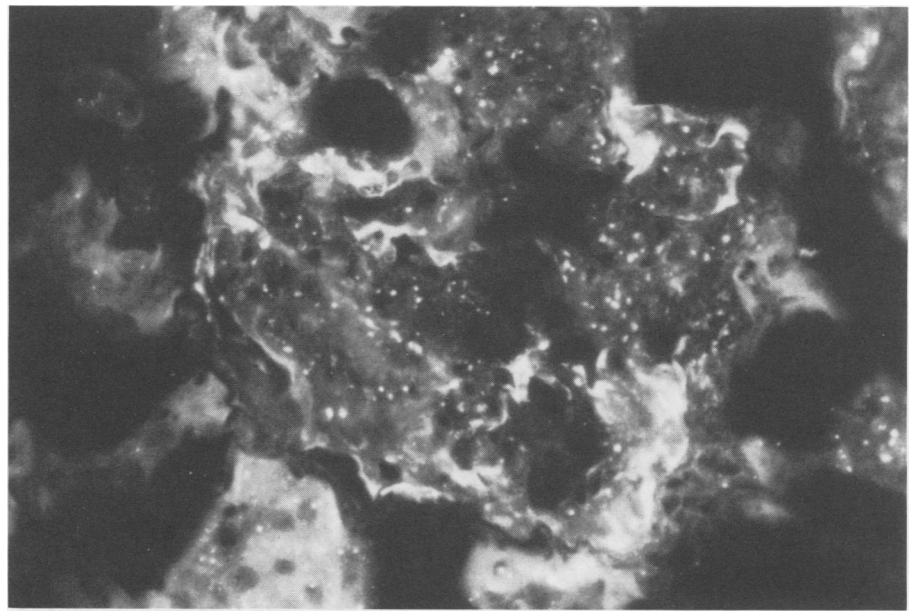

Fig. 1. Glomerulus of calf 8917 persistently infected with BVDV.

TABLE V. Calves Persistently Infected With MC Isolates of Bovine Viral Diarrhea Virus (BVDV) by Intravenous Inoculation of Pregnant Cows Seronegative for BVDV

Cow Number	Calf Number	Day of Gestation Inoculated	Appearance of Calf at Birth	Virus Isolated	Serotiter for BVDV at Birth	Disposition of Calf
30	9297	106	Normal-vigorous	Yes	Neg	Developed acute diarrhea at eight days of age and died. Fecal sample positive for BVDV, rotavirus and coronavirus
31	9298	96	Unsteady walk — stumbles — falls	Yes	Neg	Continued to stumble and fall. Died at one month of age. Cerebellar hypoplasia not demonstrated
32	9299	88	Normal-vigorous	Yes	Neg	Died at five months of age. Mild pneumonia in cardiac lobes. Ulcers in ileum and colon
33	9300	112	Normal-vigorous	Yes	Neg	Developed respiratory disease, diarrhea, and swollen stifle joint at 2.5 months of age. Did not respond to therapy — died
34	9301	72	Unsteady walk	Yes	Neg	Faltering walk became progressively worse. Necropsied at 16 days of age. Pericarditis, peritonitis, hemorrhagic ileitis and colitis. Cerebellar hypoplasia not demonstrated
35	9302	42	Normal-vigorous	Yes	Neg	Growing at six months of age — used on another experiment
36	9303	92	Normal-vigorous	Yes	Neg	Growing at six months of age — used on another experiment
37	9304	72	Normal-vigorous	Yes	Neg	Growing at six months of age — used on another experiment
38	9305	67	Normal-vigorous	Yes	Neg	Growing at six months of age — used on another experiment
39	9306	57	Normal-vigorous	Yes	Neg	Growing at six months of age — used on another experiment
40	9307	49	Normal-vigorous	Yes	Neg	Growing at six months of age — used on another experiment
41	9308	43	Very weak	Yes	Neg	Died within the hour of birth — no gross lesions
42	—	60	Aborted	ND		
43	—	80	Aborted	ND		
44	—	58	Aborted	ND		

ND = not done

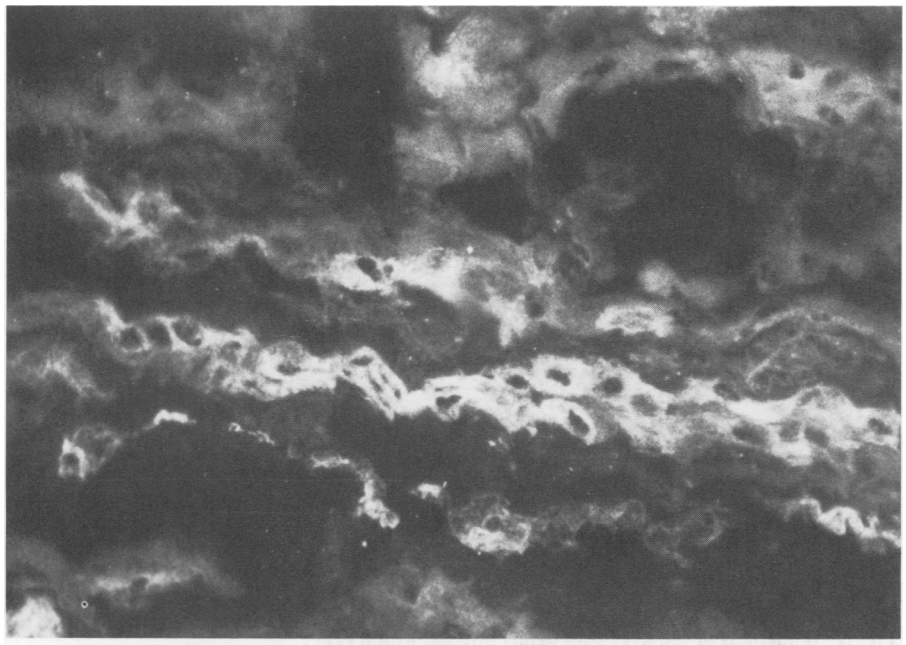

Fig. 2. Collecting tubules of calf 8917 persistently infected with BVDV.

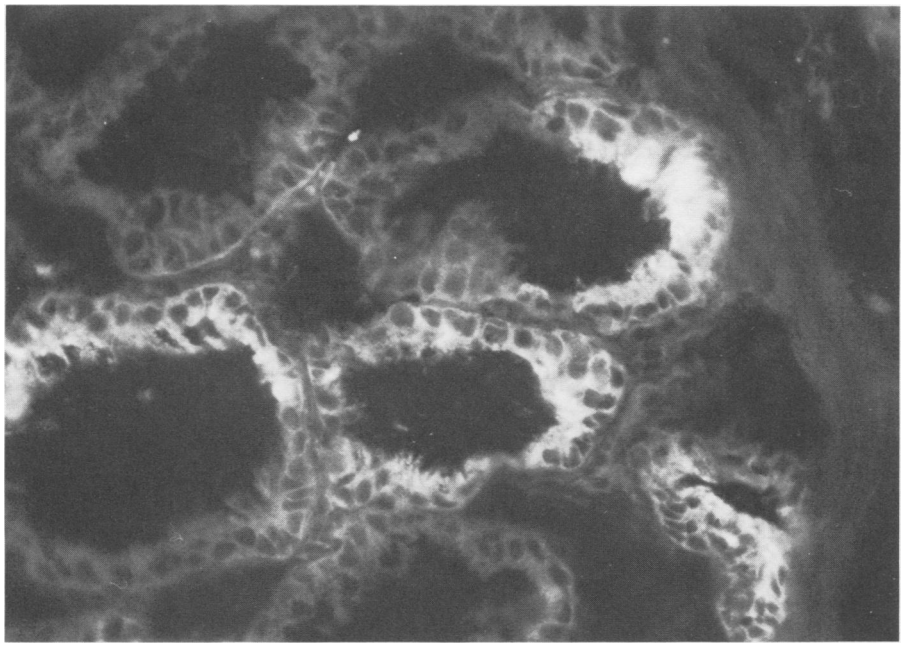

Fig. 3. Colon of calf 9301 persistently infected with BVDV.

DISCUSSION

Experiments 1 and 3 indicated when BVDV was inoculated into the amniotic fluid of fetuses between 58 and 125 days of gestation at least one third may die and be aborted or be stillborn. However, at least half may be born appearing as normal calves with no serotiters to BVDV, but in fact carrying the virus. Two apparently normal persistently infected heifers (8799 and 8875) lived to breeding age and were bred. Number 8799 has given birth to two apparently normal persistently infected calves. However, about a month after the birth of the first calf, she developed acute lameness in the left rear leg, went off feed and lost much weight. She was treated with gentamicin daily for two weeks and the calf was removed. The cow recovered and was rebred and has produced a second apparently normal persistently infected calf. Number 8875 has given birth to an apparently normal persistently infected calf and is presently doing well. Both of these animals carry BVDV isolate 7443. This state of persistent infection appears to last indefinitely and infected cows may in turn give birth to persistently infected calves.

In experiment 2, all cows inoculated intravenously with VM isolate between 49 and 84 days of gestation gave birth to normal appearing persistently infected calves. However, the viability of the persistently infected calves was no greater with the VM isolate than it was with the 7443 isolate. Only one persistently infected heifer (8911) from the VM infected group lived to breeding age. She became pregnant but lost her calf after four months pregnancy, then shortly after developed acute mucosal disease and died at two years and four months of age. One normal appearing heifer (8908) was growing very well and had no clinical signs of disease when she was killed in an accident. However, although she had no diarrhea, necropsy revealed a 30 cm portion of the ileum with marked hemorrhagic ileitis. This finding suggests that a larger portion of the intestinal mucosa must be abnormal before either hypersecretion or malabsorption will become manifest in diarrhea.

Only six of the 15 cows inoculated with the MC isolate raised apparently healthy calves to beyond eight months of age. Because of the relatively small number of cattle used in the individual experiments, two different routes of inoculation of the fetuses, and the variation in the stress on the calves resulting from different seasons and different grouping of animals, it is not possible to be certain that one BVDV isolate is more or less pathogenic than another one. However, the MC isolate differed in its effect on cattle from the VM and NADL isolates (that were also injected into BVDV seronegative cattle) in that all the cattle receiving the MC isolate developed inappetence for three days following inoculation. This clinical response indicates the MC isolate was more pathogenic at the passage level used than either the VM or the NADL isolates.

The reason for the difference between the results in experiments 2, 4 and 5, using the same route of exposure of the fetus but different BVDV

TABLE VI. Immunofluorescence of Tissues From Calves Persistently Infected With Bovine Viral Diarrhea Virus (BVDV)

Calf Number	Method of Exposure	Non-CPE Virus Isolate	Age at Necropsy	Tissues Showing Immunofluorescence
8798	*In utero* exposure of 58 day old fetus	7443	Yearling	Adrenal gland, spleen, kidney — mostly in glomeruli and basement membrane, lung, abomasum, both ulcerated and nonulcerated areas. Ileum, mesenteric lymph node of ileum and adrenal lymph node
8917	*In utero* exposure of 90 day old fetus	7443	4 months	Cerebellum, cerebrum, midbrain, adrenal, ileum (lamina propria), kidney-glomeruli and basement membrane and mesangial cells
8916	*In utero* exposure of dam 49th day of gestation	VM	Yearling	Cerebrum, corpus striatum, pyriform lobe, thalmus, spleen, kidney-glomeruli, lungs — cells in the septomesenteric lymph node, mediastinal lymph node
8913	*In utero* exposure of 98 day old fetus	7443	Stillborn	No definite fluorescence in any tissue
8912	*In utero* exposure of dam 72nd day of gestation	VM	4 months	Thymus, spleen, mesenteric lymph node
8908	*In utero* exposure of dam 79th day of gestation	VM	6 months	Cerebrum, kidney-glomeruli and basement membrane spleen, ileum
8870	*In utero* exposure of 90 day old fetus	7443	5 months	Scattered cells in perenchyma of brain stem and lamina propria of duodenum
9301	*In utero* exposure 0f 72 day fetus	MC	16 days	Abomasum, colon

isolates, cannot be determined. Although only half the volume of NADL virus was given compared to the VM and MC isolates, each mL of the NADL-BVDV contained 100 times more virus per mL. The NADL-BVDV may not have multiplied and invaded the placenta as rapidly as the VM and MC isolates. The fetuses were infected, but perhaps not until near the middle of the second trimester when the fetuses were immunocompetent. There is evidence that the bovine fetus is immunocompetent in the last half of gestation and will respond to a BVDV infection by producing antibodies and usually no evidence of disease or abortion (5).

Some of the calves in experiments 1 and 2 were stressed and lost weight and condition after weaning, and those that died did so after weaning. However, the calves that died in experiment 5 all died while still nursing their dams. In contrast to the calves of experiments 1, 2 and 5, the calves that were born with BVDV antibody (Table IV) had no health problems before or after weaning. The persistently infected calves exposed to IBR, PI-3 and three of the four exposed to *P. multocida* responded with antibody production, indicating that they are immunocompetent, but tolerant only to the isolate of BVDV that was introduced early in gestation. Therefore, the fact that some of the persistently infected calves have not done well, and even died under normal cattle rearing conditions, suggests their illness may have resulted from an additive effect of the presence of BVDV and not to a suppression of the immune system.

These experiments indicate that the birth of calves persistently infected with BVDV can result from an exposure of a BVDV negative dam to BVDV between 42 and 125 days of gestation. The vitality of these calves may be lowered and they may present a diagnostic problem. They will also be a constant source of infection for other cattle with which they may come in contact. The discovery of apparently healthy persistently infected cattle represents another facet that must be considered in the study of epizootiology and control of BVDV. The ability to produce persistently infected animals by exposing fetuses at various stages of gestation makes the development and use of an inactivated BVDV vaccine to control the disease more desirable.

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